

Appl. No. 09/577,601
Reply to Office Action of July 13, 2004

Remarks/Arguments:

According to the Office Action, mailed July 13, 2004 (hereinafter, "Office Action"), claims 4-6 and 8-12 are currently pending and under examination. In the Office Action, the Examiner made the following objections and rejections:

- The status of claims 14-43 in the Claims Listing needs to be corrected to indicate they were canceled.
- Rejection (new) of claims 5, 6 and 8-11 under 35 U.S.C. 112, second paragraph (indefiniteness).
- Rejection (new) of claim 8 under 35 U.S.C. 112, second paragraph (indefiniteness).
- Rejection (maintained) of claims 4 and 12 under 35 U.S.C. 112, first paragraph (lack of enablement).
- Rejection (maintained) of Claims 4 and 12 under 35 U.S.C. 112, first paragraph (lack of written description).
- Rejection (maintained) Claims 5, 6 and 8 under 35 U.S.C. 103(a) as being unpatentable over Bass in view of the 1998 Article and the Spaete patent.
- Rejection of claim 9 (maintained) under 35 U.S.C. 103(a) as being unpatentable over Bass in view of the 1998 Article and the Spaete patent and further in view of Barenkamp.

1. **Remarks:**

a. **Extension of time under 37 CFR 1.136(a).**

The Office Action, mailed July 13, 2004, stated that a response to it was due within three months from its mailing date, and that extensions of this time period may be granted under 37 CFR 1.136(a). Applicant attaches herewith a Petition for Extension of Time Under 37 CFR 1.136(a) requesting an extension of three months to respond and credit card payment form for the extension fee of \$1,020.00. With this extension, the time to respond is on or before January 13, 2005. As this response is filed on January 13, 2005, the response is timely filed.

b. **Amendments to the Claims.**

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Claims 4, 6 and 12 were canceled without prejudice or disclaimer.

Claims 5 and 8 were amended without prejudice or disclaimer and to further Applicants' business interests and the prosecution of the present application. Claim 5 was amended so that the term "analog" has been replaced with the term "mutant". This amendment is supported at page 11, lines 18-22 of the Instant Application. This claim was also amended to incorporate the limitation of canceled claim 6 so that the claimed nucleic acid molecule encoding a non-proteolytic mutant of a *Haemophilus* Hin47 protein includes a portion encoding the leader sequence for said non-proteolytic mutant. Claim 8 was amended so that it refers to claim 5 instead of claim 7. The amendments to the claims 5 and 8 do not add any new matter.

Applicant reserves the right to prosecute any canceled or amended subject matter in a later application.

2. Arguments.

a. Status of claims 14-43 needs to be corrected to indicate they were canceled.

The Examiner stated that the status of claims 14-43 should be "canceled" as opposed to "withdrawn" because the Amendment of December 4, 2002 canceled these claims.

Applicants thank the Examiner for pointing out that the status of claims 14-43 should have been listed as "canceled". The present Listing of Claims now reflects this.

b. Claims 5, 6 and 8-11 rejected under 35 U.S.C. 112, second paragraph.

The Examiner rejected claims 5, 6 and 8-11 under 35 U.S.C. 112, second paragraph alleging that the term "analog" is unclear.

Applicants have canceled claim 6 and so the rejection as to this claim is moot.

Applicants have amended claim 5 so that the term "analog" has been replaced with the term "mutant". With this amendment, it is clear the "non-proteolytic mutant of *Haemophilus* Hin47" of claim 5 refers to Hin47 which is mutated at amino acid 91, 121 or 197. Because claims 8-11 are either directly or indirectly dependent on claim 5, claims 8-11 are also amended by amendment of claim 5.

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Applicants respectfully request that the Examiner withdraw this rejection of claims 5, 6 and 8-11.

c. Claim 8 rejected under 35 U.S.C. 112, second paragraph.

The Examiner rejected claim 8 under 35 U.S.C. 112, second paragraph alleging that claim 8 is indefinite because the claim upon which it depends (i.e., claim 7) was canceled.

Applicants have amended claim 8 so that it refers to claim 5 instead of claim 7.

Applicants respectfully request that the Examiner withdraw this rejection of claim 8.

d. Claims 4 and 12 rejected under 35 U.S.C. 112, first paragraph.

The Examiner maintained the rejection of claim 4 under 35 U.S.C. 112, first paragraph alleging that plasmid JB-3120-2 is not enabled because U.S. Patent No. 5,506,139 (which is incorporated by reference into the instant application) does not teach the entire sequence of the plasmid identified as JB-3120-2. The Examiner also maintained the rejection of claim 12 under 35 U.S.C. 112, first paragraph alleging that the plasmid identified as DS-2342-2 is not enabled because there is no specific sequence taught for the *eco*RI T7 H91A *Hin*47 fragment that was inserted into plasmid BK-96-2-11 to form DS-2342-2.

Applicants have canceled claims 4 and 12 so the rejections are moot.

Applicants respectfully request that the Examiner withdraw these rejections of claims 4 and 12.

e. Claims 4 and 12 rejected under 35 U.S.C. 112, first paragraph.

The Examiner maintained the rejection of claim 4 and 12 under 35 U.S.C. 112, first paragraph alleging that there is no written description because the specification does not inform one skilled in the art where to acquire the plasmids used to make the claimed plasmids identified as JB-3120-2 and DS-2342-2, nor the sequences of either the claimed or used plasmids.

Applicants have canceled claims 4 and 12 so this rejection is moot.

Applicants respectfully request that the Examiner withdraw this rejection of claims 4 and 12.

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f. Claims 5, 6 and 8 rejected under 35 U.S.C. 103(a).

The Examiner maintained the rejection of claims 5, 6 and 8 as being unpatentable over Bass et al., *J. Bacteriology*, 178:1154-1161 ("Bass") in view of Loosmore et al., *Infection and Immunity*, 66(3): 899-906 (the "1998 Article") and U.S. Patent No. 5,474,914 issued to Richard Spaete (the "Spaete patent").

In the prior Office Action, dated November 17, 2003, the Examiner alleged that Bass teaches Hin47 would be useful as a chaperone protein. Bass, at page 1157, first column, said:

The HtrA/DegP family of proteins may also act as chaperones by binding periplasmic proteins that have just been secreted or those that are denatured under stressful conditions.

To support this, the Examiner cited Spiess et al., *Cell*, 97:337-347 (April 1999) ("Spiess") as teaching HtrA acts as both a chaperone and a protease and Faccio et al., *J. Biol. Chem.*, 275(4): 2581-2588 (January 2000) ("Faccio") as teaching HtrA has dual, temperature dependent functions as described in Bass. Having done this, The Examiner then alleged that the 1998 Article teaches recombinant vectors for production of non-proteolytic Hin47 analogs made by mutation of the wild type Hin47 at amino acids, 91 (H91A), 121 (D121A) and 197 (S197A). Though these references in combination were alleged to teach that a non-proteolytic form of Hin47 may be used as a chaperone protein, the Examiner admitted that they do not teach the co-expression of Hin47 with another protein. To supply what is not taught, the Examiner alleged that the Spaete patent teaches co-expression of two recombinant proteins, a chaperone protein and a second protein from a vector, provided that the chaperone is compatible with the second protein and secretion of the proteins is accomplished by including a leader sequence in the vector encoding the chaperone, the leader sequence generally being a signal peptide directing cell secretion. To combine the teaching of these references, the Examiner alleged that one of ordinary skill in the art would have known from the references above that Hin47 could be used in the disclosed vector and therefore the vector of claims 5, 6 and 8 is obvious in light of the prior art.

In the instant Office Action and in view of Applicants' arguments made in response to the prior office action, Examiner went on to allege that the Bass reference indicates that the HtrA/DegP proteins represent a family of proteins, and, as such, the teachings of this reference for one such of these proteins would be indicative of the behavior of other members of

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the family, and that the teachings of the 1998 Article, Spiess and Faccio support this view.
Having said this, the Examiner concluded:

Thus, the art recognizes a relationship among the HtrA/DepG proteins, and indicates that non-proteolytic HtrA proteins generally, and not those of any specific bacterium, would be effective as chaperones.

Applicants have canceled claim 6 and so this rejection as to this claim is moot.
Applicants respectfully traverse the rejection of claims 5 and 8 for the reasons discussed below.

Section 2142 of the MPEP states: "To establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations. The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, and not based on applicant's disclosure. *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991)."

I

Applicants present further arguments to supplement those made in the response to the Office Action, dated June 4, 2002 concerning the rejection of claims 5-8 under 35 U.S.C. 103(a).

The combination of Bass, the 1998 Article and the Spaete patent do not teach all of the elements of claims 5 or 8. In particular, this combination of references does not teach an element of the claimed expression vector - a nucleic acid molecule encoding a non-proteolytic mutant of a *Haemophilus* Hin47 protein, wherein said nucleic acid molecule includes a portion encoding the leader sequence said Hin47 protein.

The 1998 Article appears to teach a nucleic acid molecule encoding a non-proteolytic mutant of *Haemophilus* Hin47 protein without its leader sequence (i.e., a mature protein), but does not teach a nucleic acid molecule encoding such a non-proteolytic mutant of the Hin47 with its natural leader sequence. In fact, Loosmore at page 900, section titled "Expression of recombinant HtrA and generation of mutant proteins", states:

The smallest Erase-a-base clone that contained all of the *htrA* gene was used to construct expression plasmids. By analogy with the *E. coli* and *S. typhimurium*

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HtrA proteins (22), a putative 26-amino acid signal sequence was identified, and the Thr at position 27 was assumed to represent the start of the mature protein. There is a *Bsp*MI site ~72 bp downstream of the start of the coding sequence for the mature HtrA protein and *Cla*I site downstream of the end of the *htrA* gene. Oligonucleotides were synthesized to encode the N-terminus of the mature HtrA protein up to the *Bsp*MI site (Fig. 3). Plasmid DNA was digested with *Bsp*MI and *Cla*I, and the 1.4-kb fragment was ligated with the *Nde*I-*Bsp*MI oligonucleotides and vector pT7-7, which had been digested with *Nde*I and *Cla*I, thus generating pT7-7/*htrA*. For site-directed mutagenesis, the T7-*htrA* gene fragment was cloned into M13mp18 and the Amersham oligonucleotide-directed in vitro mutagenesis system was used. The T7-*htrA* mutant genes were cloned into pT7-7 for expression of the recombinant proteins.

The Spaete patent does not teach a nucleic acid molecule encoding a non-proteolytic mutant of a *Haemophilus* Hin47 protein with its leader sequence. Instead, the Spaete patent is specifically concerned with secreting truncated cytomegalovirus glycoprotein using suitable fibroblast growth factor receptor as the chaperone and the claims of the patent, as well as its teachings, are so limited. Further, the Spaete patent does not teach the use of natural leader sequence of the escort (or chaperone) protein, but instead teaches at col. 21, lines 44-54:

Alternatively, foreign proteins can also be secreted from the cell into the growth media by creating chimeric DNA molecules that encode a fusion protein comprised of a signal peptide sequence fragment that provides for secretion in yeast of the foreign protein. Preferably, there are processing sites encoded between the leader fragment and the foreign gene that can be cleaved either in vitro or in vivo. The leader sequence fragment typically encodes a signal peptide comprised of hydrophobic amino acids which direct the secretion of the protein from the cell. [underline added for emphasis]

The fact that the DNA is described to be chimeric (col. 21, line 46) suggests that the leader sequence associated with the escort (or chaperone) protein is not its natural leader sequence, but rather one from a foreign source.

Bass does not teach a nucleic acid molecule encoding a non-proteolytic mutant of a *Haemophilus* Hin47 protein with its leader sequence. Instead, Bass appears to teach the isolation and characterization of two novel putative protease genes of *E. coli*, *hhoA* and *hhoB*, which are said to be found in *E. coli* and homologous to HtrA of *E. coli*. Bass does not teach anything about *Haemophilus* Hin47 proteins.

In conclusion, as neither Bass nor the 1998 Article nor the Spaete patent teach a nucleic acid molecule encoding a non-proteolytic mutant of the *Haemophilus* Hin47 protein with its leader sequence, the combination of these references cannot teach such a nucleic acid molecule. For this reason, the combination of Bass, the 1998 Article and the Spaete patent does

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not teach a limitation of claims 5 or 8; and, therefore, the claims would not be obvious under 35 U.S.C. 103(a).

II

The teachings of Bass, as supplemented by teachings of either the 1998 Article, Spiess or Faccio, do not provide a reasonable expectation of success in the use of a recombinant non-proteolytic mutant Hin47 protein as a chaperone protein. Put differently, these references do not indicate that non-proteolytic HtrA proteins generally, and not those of any specific bacterium, would be effective as chaperones.

First, the Examiner alleges that 1998 Article supports the teaching of Bass that the recombinant non-proteolytic mutant Hin47 protein disclosed in the Instant Application is a chaperone protein. Specifically, the Examiner alleges that the 1998 Article, at pages 901-902, demonstrates similarities between the activity of the *E. coli* and *Haemophilus* versions of the HtrA/DepG protein and thereby highlight the relationship between the two proteins. The Applicants respectfully note that 1998 Article, at pages 901-902, appears to discuss various biochemical properties of the Hin47 protein - it has a molecular mass of 49.2 kDa, and has 54% identity and 69% similarity to the *E. coli* and *S. typhimurium* HtrA proteins; it is a stress response protein behaving similarly to *E. coli* HtrA in culture at an elevated temperature (43.5°C) or in the presence of 6% ethanol; it has serine protease activity; it has a catalytic triad associated with other bacterial or mammalian serine proteases; and, unlike, the *E. coli* and *S. typhimurium* HtrA proteins, it does not contain any cysteine residues. The mere comparison of the biochemical properties of *Haemophilus* Hin47 protein and *E. coli* and *S. typhimurium* HtrA proteins suggesting similarities between the proteins may suggest to one with ordinary skill in the art that the Hin47 may be a stress response protein like *E. coli* and *S. typhimurium* HtrA, but would not suggest that the Hin47 protein is a chaperone. In fact, the 1998 Article, starting at the last sentence on page 899, first column, highlights a biochemical difference (thermal stress response) between *E. coli* and *S. typhimurium* HtrA proteins by stating:

The *S. typhimurium* HtrA protein is ~89% identical to *E. coli* HtrA but is not induced by heat shock, although it is induced by oxidative stress.

This would serve to highlight that differences between the two HtrA proteins exist, and would raise a question in the mind of the person with ordinary skill in the art as to what other biochemical properties (as, for example, the chaperone-like property) would differ between *E. coli* HtrA and *Haemophilus* Hin47, even though they are said to be related.

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Second, as noted above, the Examiner alleges that Spiess supports the alleged teaching of Bass that the recombinant non-proteolytic mutant Hin47 protein disclosed in the Instant Application is a chaperone protein. Specifically, the Examiner alleges that Spiess, at the abstract and page 339, right paragraph, discusses the HtrA/DepG proteins generally and indicates that the DepG protein is present in multiple bacterial species. Spiess, in pertinent part, said:

DegP is part of large family of serine proteases, members of which are found in most organisms, including humans. Bacterial DegP has been implicated in thermal, osmotic, and pH tolerance and H₂O₂ resistance. In addition, DegP seems to be involved in bacterial virulence.

First, the Applicants respectfully note that this passage of Spiess, in the context of the remainder of the reference, is directed to the DepG (or HtrA) protein from *E. coli*, not Hin47 from *Haemophilus*. In particular, Spiess at page 339, first column, Summary, in pertinent part, states that its findings are clearly made with respect to the DegP (HtrA) protein of *E. coli*, not the large family of proteases of which DegP is member.

We show here that the widely conserved heat shock protein DegP (HtrA) has both general molecular chaperone and proteolytic activities.

Because the teaching of Spiess is stated in the singular (as opposed to plural) and because it limited to DegP (HtrA) of *E. coli*, the allegation that Bass (as supported Spiess) teaches that the recombinant mutant non-proteolytic Hin47 protein disclosed in the Instant Application is chaperone protein does not follow. Also, the passage of Spiess cited by the Examiner appears to teach that there exists large family of proteins which have the biochemical properties of being a serine protease and which may aid bacterial organisms in resistance to thermal, osmotic or pH shock, or H₂O₂. Neither this passage nor Spiess in total establish that one such property of this large family is a chaperone-like activity.

Third, as noted above, the Examiner alleges that Faccio supports the alleged teaching of Bass that the recombinant non-proteolytic mutant Hin47 protein disclosed in the Instant Application is a chaperone protein. Specifically, the Examiner alleges that Faccio, at the abstract and pages 2581 and 2585-2586, generally discusses "bacterial HtrA", and the discussion is not limited to discussion of the *E. coli* protein. The Applicants respectfully note that in each instance cited by the Examiner in Faccio, the term "bacterial HtrA" is discussed in the singular versus plural, suggesting that this term is referring only to the DepG (HtrA) protein of *E. coli*. The abstract of Faccio, in pertinent part, states:

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Omi protein consists of 458 amino acids has homology to bacterial HtrA endoprotease...

Homology is generally defined with respect to single reference protein. Page 2581, second column of Faccio, in pertinent part, states:

It has been recently found that bacterial HtrA has a dual role acting as a chaperone at normal temperatures and an active protease at elevated temperatures (7).

The reference (7) is Spiess, the teaching of which are discussed above. Finally, pages 2585-2586, in pertinent part, states:

We describe the isolation and characterization of Omi, a human homologue of the bacterial HtrA endoprotease. Prokaryotic heat shock proteins (HtrAs) are enzymes activated by high temperature that remove denatured or damaged proteins from heat-stressed bacteria (23-26). The importance of HtrAs became apparent when it was learned that bacteria lacking this enzyme are unable to survive at 42°C (24, 26). Furthermore, pathogenic bacteria that lack HtrA are less virulent suggesting that Omi may have a role in counteracting and neutralizing host bactericidal mechanisms (3). Recently, it has been reported that bacterial HtrA has a dual role, acting as a chaperone at normal temperatures and as an active protease against a natural substrate (the MalS protein) at elevated temperature (7).

As noted above, the reference (7) is Spiess, the teaching of which are discussed above. Applicants respectfully note that this passage of Faccio appears to teach that HtrAs are enzymes activated by high temperature that remove denatured or damaged proteins from heat-stressed bacteria, but does not teach that Omi specifically or bacterial HtrAs generally are chaperones. The reference to "bacterial HtrA" appears to be in the singular as opposed to plural because it is referenced to Spiess.

In conclusion, neither the 1998 Article, Spiess nor Faccio support the alleged teaching of Bass that the recombinant non-proteolytic mutant Hin47 protein disclosed in the Instant Application is a chaperone protein. This because Bass (in combination with the 1998 Article, Spiess and Faccio) only teach that there is one member, DepG (HtrA) protein of *E. coli*, is chaperone, and do not teach that any *Haemophilus* Hin47 protein is a chaperone. Accordingly, the combination of Bass (as said to be supported by the 1998 Article, Spiess and Faccio), the 1998 Article and the Spaete patent provide no reasonable expectation of success in using the

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recombinant non-proteolytic mutant Hin47 protein disclosed in the Instant Application as a chaperone protein. For this reason, claims 5 and 8 would not be obvious under 35 U.S.C. 103(a).

III

As shown above, the combination of Bass, the 1998 Article and the Spaete patent do not teach all of the elements of claims 5 or 8 because none of the references teach the element of the claimed expression vector - a nucleic acid molecule encoding a non-proteolytic mutant of a *Haemophilus* Hin47 protein, wherein said nucleic acid molecule includes a portion encoding the Hin47 leader sequence. Furthermore, the combination of Bass (as said to be supported by the 1998 Article, Spiess and Faccio), the 1998 Article and the Spaete patent do not provide a reasonable expectation of success of using the recombinant non-proteolytic mutant Hin47 protein disclosed in the Instant Application as a chaperone protein. For these reasons, the Examiner has not established a *prima facie* case of obviousness for claims 5 and 8. Accordingly, Applicants respectfully request that the Examiner withdraw this rejection as to claims 5 and 8.

g. Claim 9 rejected under 35 U.S.C. 103(a).

The Examiner maintained the rejection of claim 9 under 35 U.S.C. 103(a) as being unpatentable over Bass in view the 1998 Article and the Spaete patent and further in view of Barenkamp and St. Geme III, *Molecular Microbiology*, 19: 1215-1223 ("Barenkamp").

The inclusion of the teaching of Barenkamp with the teachings of Bass, the 1998 Article and the Spaete patent does not assist in the establishment a *prima facie* case of obviousness under 35 U.S.C. 103(a) because, as discussed above, the combination of Bass, 1998 Article and the Spaete patent do not teach all elements of the expression vector of claim 5 (on which claim 9 depends), nor do these references establish a reasonable expectation of success that the recombinant non-proteolytic mutant Hin47 protein disclosed in the Instant Application is a chaperone protein. Thus, the Examiner cannot establish a *prima facie* case of obviousness under 35 U.S.C. 103(a) as to claim 9. Accordingly, Applicants respectfully request that the Examiner withdraw this rejection as to claim 9.

3. Conclusions.

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The amendments, remarks and arguments submitted herein are intended to be fully responsive to the outstanding Office Action, to advance the prosecution of the present invention, and to place the application in condition for allowance.

The Applicants respectfully requests consideration and entry of this paper. The Applicants also respectfully requests reconsideration of this application, as amended, and issuance of a timely Notice of Allowance in this case. Should the Examiner have any questions concerning this application, she is invited to contact the undersigned at (570) 839-5537. If necessary, please charge any additional fees required or credit any fees overpaid to Deposit Account No. 50-0244.

Respectfully submitted,

Date: January 13, 2005

By: Robert Yoshida

Robert Yoshida
Reg. No 54,941

Aventis Pasteur, Inc.
Intellectual Property - Knerr Building
One Discovery Drive
Swiftwater, PA 18370
Telephone: (570) 839-5537
Facsimile: (570) 895-2702